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Glomerular filtration effects of acute volume expansion: Importance of chloride

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Glomerular filtration effects of acute volume expansion: Importance of chloride. The present studies were done to determine the effect on GFR of acute volume expansion (AVE) using solutions of various sodium salts and to explore if degree of tubuloglomerular feedback (TGF) activation plays a role in any GFR differences. Free-flow micropuncture and inulin clearance studies were combined to investigate anesthetized Munich-Wistar rats expanded to 10% body weight with isotonic solutions of NaCl, Ringers bicarbonate (RB), $NaHCO_3$, Na acetate (NaAc) and Na_2SO_4 as well as euvolemic controls. In the clearance studies, AVE yielded per gram kidney weight GFR's greater than control (1009 ± 51 μl/min) in the NaCl and RB (chloride expanded) groups (1397 ± 89 and 1389 ± 64) μl/min, respectively, $P < 0.05$ *vs.* control) but not in the $NaHCO_3$, NaAc, and Na_2SO_4 (non-chloride expanded) groups. Proximal minus distal single nephron GFR determinations (P-D), an estimate of the degree of TGF, were less than control 13.2 ± 2.1 nl/min) in the NaCl and RB groups (4.1 ± 0.7 and 5.3 ± 1.9 nl/min, respectively, $P < 0.05$ vs. control) but were not significantly different from control in any of the non-chloride expanded groups. Early distal (ED) fluid flow correlated positively with P-D in all groups. ED chloride concentration but not TCO_2 nor osmolality correlated with P-D for all groups. The correlation was negative for control and chloride expanded groups and positive for non-chloride expanded groups. We conclude 1.) AVE with chloride, but not non-chloride, solutions results in whole kidney GFR's greater than control due at least in part to greater inhibition of TGF by chloride expansion; 2.) ED chloride is correlated with opposite effects on TGF in chloride versus non-chloride AVE. We propose that this difference in TGF activation relates to characteristic intrarenal hormonal environments created by these different models of AVE in which the TGF mechanism is enacted.

Intravenous isotonic sodium salt-solutions are given for both experimental and therapeutic purposes. Little data exists detailing the effect on glomerular filtration of acute infusions of various sodium salts, but there is suggestion that each salt may have characteristic effects. Two studies involving the same volume infusions of equimolar sodium bicarbonate and sodium chloride solutions yielded lower per kilogram body weight GFR's in the bicarbonate infused compared to the chloride infused animals [1, 2]. Since the animals were similarly volume expanded, the difference in GFR's may have resulted from intrarenal factors. One intrarenal factor that may play a role is tubuloglomerular feedback (TGF).

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TGF has been shown to be stimulated by plasma volume or extracellular volume (ECF) depletion [3–5] and inhibited in states of ECF volume expansion [3, 6, 7]. Thus, TGF probably helps the kidney in maintaining ECF volume homeostatis. Nevertheless, factors other than volume may be important in mediating TGF since the exact sensing signal(s) for tubuloglomerular feedback (TGF) remains unclear. Increase in tubular fluid flow past the macula densa has been shown by many investigators to initiate TGF [6–12], but more recent studies have focused on ion content of this fluid as being a signal. Evidence from in vivo microperfusion studies support distal nephron tubular chloride concentration and/or its transport as being at least one signal [13, 14], while other studies support an important role for distal tubule fluid osmolality [15, 16]. Other investigators have suggested that both of these parameters may work in concert to stimulate TGF [17].

Controversy also exists concerning the effector limb(s) of the TGF mechanism, but many investigators feel that part of this mechanism may involve local alteration in synthesis and/or release of intrarenal hormones [18, 19]. If this is the case, then baseline hormonal status may influence the ultimate outcome of the TGF response initiated by the signals (or others) described above.

The present studies were undertaken to investigate the effect of intravenous infusion of sodium salts of different anions on GFR. Free-flow micropuncture and clearance studies were employed. Differences in late proximal and early distal tubule SNGFR determinations were used as an estimate of the degree of TGF and an attempt was made to correlate this difference with distal fluid anion content and osmolality. The data demonstrate the importance of chloride in the infusion solution in determining the whole kidney GFR response to sodium salt volume expansion. In addition, the data suggests that early distal chloride concentration acts as a TGF signal independent of early distal fluid flow in influencing TGF. Finally, it is proposed that characteristic intrarenal environments resulting from volume expansion using these different sodium salts contribute to the final TGF response initiated by a given early distal tubule signal.

Methods

Munich-Wistar rats (200 to 250 g) maintained on a standard Ralston Purina rat chow (Ralston Purina Co., St. Louis, Missouri, USA) diet and tap drinking water were used in these

studies. The animals were anesthetized with an intraperitoneal injection of Inactin (100 mg/kg body wt) (BYK, Hamburg, West Germany), a tracheal tube inserted and the animal allowed to spontaneously breathe room air. PE50 tubing was inserted into the right jugular vein and the right femoral artery for infusion of solutions, monitoring of blood pressure, and sampling of arterial blood, respectively. A left flank incision was made to expose the left kidney which was gently mobilized from its surrounding tissue and adrenal gland. The kidney was then placed in a Lucite cup, further stabilized with 2% agar and bathed by a constant drip of warm, water–equilibrated mineral oil. After baseline arterial blood gases and chemistries were obtained, the various solutions detailed below were infused 10% body weight over one hour (loading solution) followed by 5.0% body weight/hour (maintenance solution) for the remainder of the experiment. Control animals were replaced with a simulated plasma solution (Ringers bicarbonate plus 40 g/liter bovine serum albumin) 1% body weight followed by 1% body weight/hour. This protein concentration was chosen to match the plasma protein concentration of the control animals. After the infusion of the loading solution was complete, a 1 ml bolus of 100 μCi/ml ^{3}H-inulin (New England Nuclear, Boston, Massachusetts, USA) was infused followed by the infusion of 100 μ Ci/hour dissolved in the maintenance solution.

After a one–hour inulin equibration period, two thirty–minute micropuncture periods were done using washed glass pipets filled with Sudan black–stained, water–equilibrated mineral oil. Pipets of outer diameter of 8 to 10 μm were used for proximal and of 6 to 8 μm for distal tubule punctures. Proximal and distal tubules were localized by observing the transit of 0.02 to 0.05 ml bolus injections of 2% F D and C green dye. Animals with proximal tubule transit–times of greater than fifteen seconds, distal retention of F D and C green dye, or mean arterial pressure below 100 mm Hg were discarded. Free–flow micropuncture samples were obtained from the last accessible proximal and first accessible distal tubular sites in an unpaired manner. Late proximal tubules were localized as the last tubules to disappear from the kidney surface after the first or proximal phase of F D and C dye injection. After insertion of the pipet into the tubule, a small drop of Sudan black–stained mineral–oil was injected and the tubule used for collection of fluid only if the drop immediately disappeared into the interior of the kidney. Early distal tubules were localized as the first to appear on the kidney surface after the "loop phase" of F D and C green injection, which was followed by the appearance of distal tubules stained with F D and C green dye. Tubular collections were performed as follows: a small drop of Sudan black–stained, water–equilibrated mineral oil was injected into the tubule to determine the direction of flow. Then, a column of oil 3 to 5 tubular diameters in length was injected and a timed tubular fluid collection was obtained, aspirated so as to maintain the injected oil column in a stationary position. Collections during which the injected oil column could not be kept stationary or more than minimum negative pressure was required to aspirate the fluid were discarded. Fluid collections were obtained over a period of two to three minutes for proximal and three to five minutes for distal samples. Two to four tubular collections at each site were done per animal. Fluid samples were deposited under water–equilibrated mineral oil and aliquots immediately taken for measurement of inulin and total CO_2 concentration. Subsequently, aliquots for measurement of chloride concentration and osmolality determinations were analyzed on the same day of the experiment. Total volume of the sample was determined by using previously calibrated, constant bore pipets. Urine was collected during each of the two micropuncture periods under water–equilibrated oil in tared vials, and mid-period blood samples from the tail vein were obtained for inulin clearance determinations. Arterial blood (0.35 ml) was obtained at the beginning of each micropuncture period for determination of pH, PCO_2 and electrolytes and replaced with an equivalent amount of simulated plasma.

The following groups of animals were studied.

Group I (control). Eight animals had their surgical losses replaced with simulated rat plasma. The infusion of 1% body weight bolus followed by 1% body wt/hour continuous infusion maintained hematocrit and serum protein unchanged from baseline values.

Group II (NaCl). Twelve animals were infused as described with a loading solution consisting of (in mM) NaCl 140, KCl 5, $CaCl_2$ 2 and maintenance solution of NaCl 135, KCl 15, and $CaCl_2$ 4.

Group III (RB). Ten animals were infused with a loading solution (in mM) of NaCl 114, $NaHCO_3$ 25, KCl 5, $CaCl_2$ 2; the maintenance solution was NaCl 114, $NaHCO_3$ 25, KCl 15, and $CaCl_2$ 4.

Group IV ($NaHCO_3$). Twelve animals were infused with a loading solution (in mM) of $NaHCO_3$ 145, $KHCO_3$ 10. The maintenance solution consisted of $NaHCO_3$ 140, $KHCO_3$ 15. In addition, a solution of 150 mM calcium acetate was infused via a separate vein at a rate of 0.075 mM/hr to replace calcium losses.

Group V (NaAc). Twelve animals were infused with a loading solution (in mM) of NaAc 140, KAc 5, CaAc 2. The maintenance solution was NaAc 135, KAc 15, and CaAc 4.

Group VI (Na_2SO_4). Eleven animals were infused with a loading solution (in mM) of Na_2SO_4 90, K_2SO_4 5, $CaSO_4$ 4. The maintenance solution was Na_2SO_4 90, K_2SO_4 10, and $CaSO_4$ 4.

For each of the groups described above, separate inulin clearance studies were done in five animals without micropuncture. After volume expansion as described above, inulin was infused as above but at a lower dose (20 μCi/hr) and allowed one hour equilibration, following which the clearance determinations were done. Two fifteen–minute periods were done with the animal intact, followed by two periods after exposure of the kidney as described for micropuncture. Finally, two periods were done after stabilization of the kidney in the Lucite cup.

Balance studies to determine total body retention of fluid and sodium during infusion of the experimental solution were done for all animals.

Analytical methods

Immediately after termination of the experiment, measured portions of tubular fluid samples (15 nl), tail vein plasma (1 μl), and urine (1 μl) were deposited in 5 ml of Scint Verse (Fisher Scientific Co., Fairlawn, New Jersey, USA) and counted on a Tri-carb liquid scintillation counter (Packard Instruments, Downers Grove, Illinois, USA) for determination of ^{3}H inulin activity. Urine and arterial blood pH and PCO_2 were determined immediately after collection in a blood gas analyzer (Radiometer Co., Copenhagen, Denmark). Urine and plasma

Table 1. Plasma values

	Hct %	Pro g/dl	Na^+ mEq/liter	K^+ mEq/liter	CA^{++} ionized mEq/liter	Cl^- mEq/liter	HCO_3^- mEq/liter	Osm mOsm/liter	pH	PCO_2 mm Hg
Control	48.6	3.93	149	3.86	2.89	114	22.8	292	7.361	41.7
$N = 65$	± 0.4	± 0.09	± 0.8	± 0.15	± 0.15	± 1.3	± 0.5	± 2.7	± 0.01	± 1.4
Control, Exp	48.3	3.87	155	3.76	3.30	116	22.1	299	7.374	39.3
$N = 8$	± 0.7	± 0.13	± 1.0	± 0.14	± 0.15	± 1.1	± 0.8	± 2.6	± 0.03	± 2.3
NaCl	44.3	2.50	154	4.23	3.05	123	16.5	285	7.309	34.4
$N = 12$	± 0.9[a]	± 0.06[a]	± 2.1	± 0.31	± 0.25	± 2.2[a]	± 1.1[a]	± 2.9	± 0.06[a]	± 3.5
RB	47.8	3.18	147	4.39	3.47	117	21.1	290	7.376	37.1
$N = 10$	± 0.6[b]	± 0.19[a]	± 2.4	± 0.47	± 0.36	± 4.3	± 4.3	± 1.9	± 0.02	± 1.4
$NaHCO_3$	45.5	2.52	151	3.54	3.18	96.0	42.6	277	7.607	44.5
$N = 12$	± 1.1[a]	± 0.02[a]	± 1.6	± 0.33	± 0.28	± 1.5[a]	± 3.1[a]	± 2.8[a]	± 0.01[a]	± 1.8
NaAc	45.3	2.68	146	4.07	3.24	79.6	35.3	270	7.577	41.1
$N = 12$	± 0.4[a]	± 0.15[a]	± 1.9	± 0.62	±0.24	± 0.2[a]	± 5.4	± 9.3	± 0.01[a]	± 2.1
$NaSO_4$	45.8	2.68	153	4.28	3.07	98.9	16.4	283	7.307	33.9
$N = 11$	± 0.8[a]	± 0.06[a]	± 1.7	± 0.38	± 0.15	± 2.8	± 1.0[a]	± 6.2	± 0.06	± 3.6

[a] $P < 0.05$ vs. control
[b] different by paired t-test

sodium and potassium concentrations were measured by flame photometry (IL Autocal flame photometer, Instrumentation Laboratory Inc, Lexington, Massachusetts, USA). Urine and plasma chloride concentrations were determined using a chloridometer (Corning Medical and Scientific, Medfield, Massachusetts, USA). Plasma protein was determined by refractometry. Urine and plasma osmolality were measured by a vapor pressure osmometer (Wescor Inc., Logan, Utah, USA). Plasma and urine ionized calcium were measured with an ionized calcium analyzer (Radiometer Model ICA1, Copenhagen, Denmark).

Tubular fluid samples were deposited on a siliconized glass slide under water and 100 mM Hepes (N-2 hydroxyethylpiperazine-N-2 ethanesulfonic acid) buffer solution–equilibrated mineral oil. An 8 nl aliquot was taken immediately after deposition on the slide for total CO_2 measurement (done in duplicate) performed by microcalorimetry (picapnotherm, model GV-1, WPI Instruments, New Haven, Connecticut, USA), as described by Vurek, Warnock and Corsey [20]. Total CO_2 in a sample represents bicarbonate plus the dissolved CO_2 gas. Under the present experimental conditions the total CO_2 concentration was taken to represent the bicarbonate concentration in the sample. A 1 nl aliquot of tubular fluid was taken for chloride concentration determined (in triplicate) by the micromethod of Ramsey, Brown and Croghan [21]. A 4 nl aliquot was taken for measurment of osmolality on a Clifton nanoliter osmometer (Clifton Technical Physics, Hartford, New York, USA) using a sterling silver sample plate.

Calculations

Whole kidney GFR was calculated using the clearance of inulin. Single nephron, glomerular filtration rate was calculated by multiplying the tubular fluid–to–plasma inulin–concentration–ratio times the tubular flow rate. The tubular flow was determined by dividing the total collected tubular volume by the time of collection. All whole kidney and single nephron GFR's were expressed per gram kidney weight. This was done to remove the contribution of increasing kidney weight [22] and animal age [23] to superficial nephron, single nephron glomerular filtration rate of these relatively young rats. Fractional electrolyte excretions were calculated using urine flow from both kidneys of the micropunctured animals using standard formulae. The two to four SNGFR determinations for each site in each animal were meaned and a single value obtained for each animal at each site. The difference between these two values represented the P-D for that animal. Absolute chloride delivery was calculated by multiplying the tubular flow times the tubular concentrations. Loop segment chloride–reabsorption was the difference between late proximal and early distal deliveries. For determination of loop segment chloride–reabsorption, the late proximal flow–rate used was calculated by dividing the early distal SNGFR for that animal by the animal's mean tubular fluid to plasma inulin ratio for the late proximal tubule of that animal. This calculated and not the measured late proximal flow–rate was considered a more accurate representation of the in situ flow rate because of the tubuloglomerular feedback–mediated increase in proximal tubule fluid flow produced by the discontinuation of loop of Henle fluid–flow during proximal tubule fluid–collections [11]. This calculation assumes maintenance of glomerular tubular balance for fractional fluid reabsorption with changes in proximal tubule flow–rate. Other investigators have shown that changes in proximal tubule flow–rate produced by changing loop of Henle flow rate using native tubule fluid result in maintained fractional proximal tubule fluid reabsorption [24], supporting this method of estimating in situ proximal tubule flow. Retained fluid and sodium was determined by subtracting the total amount lost through the urine from both kidneys and arterial sampling from the total amount infused. Results were corrected for 100 g body weight. Urine and blood bicarbonate concentrations were calculated from the pH and PCO_2 according to the Henderson–Hasselbalch equation. A pK of 6.1 was used for blood. A pK of 6.33 to $0.5\sqrt{C}$ was used for urine where C represents the total cation concentration estimated as the sum of sodium plus potassium expressed in equivalents per liter.

Results were expressed as mean ± SE. The results of the individual tubules were averaged for each animal for each puncture site. Statistical significance was determined using Student's t-test for paired or unpaired observations where

Table 2. Urine data

	$\dot{V}$ *μl/min/g kid wt*	U_{Na} *mEq/liter*	FE_{Na} %	U_K *mEq/liter*	FE_K %	$U_{Ca^{++}}$ *ionized mEq/liter*	$FE_{Ca^{++}}$ %	U_{Cl} *mEq/liter*	FE_{Cl} %	U_{HCO_3} *mEq/liter*	FE_{HCO_3} %
Control	3.2	528	1.6	62	7.8	—	—	281	1.7	151	3.0
$N = 8$	± 0.46	± 97	± 0.3	± 10	± 0.9			± 30[b]	± 0.1	± 20[b]	± 0.2
NaCl	35.1	177	4.4	64	52	3.5	9.7	248	7.2	0.26	0.05
$N = 12$	± 4.2[a]	± 8.5[a]	± 0.6[a]	± 7.6	± 2.6[a]	± 0.4	± 0.1	± 16	± 0.8[a]	± 0.1[a]	± 0.02[a]
RB	46.0	140	4.3	141	166	3.0	8.0	267	10	1.1	0.2
$N = 10$	± 7.4[a]	± 9.2[a]	± 0.8[a]	± 18[a]	± 38[a]	± 0.5	± 0.9	± 13	± 1.7[a]	± 0.6[a]	± 0.1[a]
$NaHCO_3$	50.3	167	7.4	39	73	2.7	10	14	1.1	120	11
$N = 12$	± 6.7[a]	± 2.7[a]	± 1.3[a]	± 3.7[a]	± 3.4[a]	± 0.3	± 0.1	± 3.5[a]	± 0.5	± 6.7	± 1.1[a]
NaAc	40.5	207	5.4	60	65	2.0	8.6	15	1.0	127	13
$N = 12$	± 5.3[a]	± 18[a]	± 0.7[a]	± 16	± 2.7[a]	± 0.3	± 0.8	± 3.3[a]	± 0.3	± 19	± 1.1[a]
Na_2SO_4	40.1	275	11	53	74	7.8	11	7.7	0.5	0.19	0.03
$N = 11$	± 4.6[a]	± 24[a]	± 0.8[a]	± 6.9	± 7.7[a]	± 0.8	± 0.1	± 1.9[a]	± 0.1[a]	± 0.10[a]	± 0.02[a]

[a] $P < 0.05$ vs. control
[b] done using micromethod described for tubular samples

Table 3. Whole kidney GFR (μl/min/g kidney wt) before and after flank incision [GFR(FI)] and after left kidney placed in Lucite cup [GFR(FIC)]

	N	GFR	GFR (FI)	% vs. control	GFR (FIC)
Control	5	1009 ± 51	723 ± 85[b]	28.1	779 ± 81
NaCl	5	1397 ± 89[a]	972 ± 53[a,b]	30.4	950 ± 55
RB	5	1389 ± 64[a]	1008 ± 53[a,b]	27.4	1001 ± 50
$NaHCO_3$	5	1054 ± 58	997 ± 91[a]	—	990 ± 87
NaAc	5	998 ± 61	912 ± 87[a]	—	920 ± 88
$NaSO_4$	5	704 ± 41[a]	691 ± 31	—	680 ± 40

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ GFR (FI) vs. GFR
% vs. control = GFR − GFR (FI)/GFR × 100

appropriate. Analysis of variance was used when more than two means were compared.

Results

Table 1 depicts the plasma parameters determined in the micropunctured animals. The basline values from all the animals were included as control, while the subsequent values denote those measured after infusion of the different experimental solutions. The statistical comparisons for the volume expanded groups were with the experimental period of the control animals ($N = 8$) and not with the control period for the total number of animals ($N = 65$). Not depicted are the mean systemic blood–pressures that were not different between groups. There were the expected changes in hematocrit and serum protein in these significantly volume–expanded animals. Neither absolute nor percentage decrease from the respective initial value were signficantly different for blood pressure, hematocrit, and serum protein concentration among volume expanded groups. As was the design of the protocols, serum potassium and ionized calcium were maintained statistically no different from controls.

Table 2 depicts the urinary data. For the control animals, urine chloride and bicarbonate was measured by the micromethods described because of the small volumes of urine produced by these animals. Also because of the small control urine volumes, measurements of urinary ionized calcium of control animals were not done. Urine volumes, FE_{Na}, and FE_K were as expected. The high $FE_{Ca^{++}}$ reflected the supplemental calcium given to maintain normal serum levels. Chloride was vigorously excreted in the chloride expanded animals and conserved in the non-chloride expanded groups. Bicarbonate was excreted in those animals loaded with bicarbonate or its substrate and conserved in the non-bicarbonate loaded animals.

Table 3 depicts the results of the inulin clearance studies. For the intact animals, there was a significant increase in whole kidney per gram kidney weight GFR as compared to control (1009 ± 51 μl/min) in the NaCl and RB expanded groups (1397 ± 89 and 1389 ± 564 μl/min, respectively, $P < 0.05$ vs. control) but there was no increase compared to control in the animals expanded with the remaining sodium salts. After flank incision (FI), whole kidney GFR was higher than control for all the expanded groups except Na_2SO_4. FI resulted in a significant decrease in whole kidney GFR compared to the intact animal in control, NaCl, and RB groups (28.1, 30.4, and 27.4% decrease, respectively) but resulted in no change in GFR for the remaining groups. Stabilization of the kidney in the lucite cup had no effect on GFR in any group. The Na_2SO_4 group was the only expanded group to have a whole kidney GFR less than control in the intact animals.

The micropuncture data are depicted in Table 4. The single nephron glomerular filtration rate (SNGFR) per gram kidney weight measured at the early distal tubule was higher than control in all the volume expanded groups. The SNGFR measured at the proximal tubule was statistically the same for all groups. The late proximal tubule flow rates depicted in Table 4 are the measured and not the calculated value used subsequently for calculation of loop segment chloride–reabsorption (**Methods**). The difference between SNGFR determined at the proximal and distal tubule (P-D), an estimate of the degree of TGF was less than control (13.2 ± 2.1 nl/min/g kidney wt) in the NaCl and RB groups (4.1 ± 0.7 and 5.3 ± 1.9 nl/min/g kidney wt, respectively, $P < 0.05$ vs. control) but not different from control in the remaining groups. The tubular fluid bicarbonate concentrations were as expected. Early distal tubule chloride–concentration was greater than control in the chloride expanded

Table 4. Micropuncture data

	Loc	$(TF/P)_I$	Flow *nl/min/g kid wt*	SNGFR *nl/min/g kid wt*	P-D *nl/min/g kid wt*	TCO_2 *mEq/liter*	Cl *mEq/ liter*	Osm *mOsm/liter*
Control	LP	2.2 ± 0.12	17.2 ± 1.8	38.1 ± 2.6	13.2 ± 2.1	7.6 ± 1.0	126 ± 3.4	291 ± 3.2
$N = 8$	ED	4.9 ± 0.54	5.3 ± 0.7	25.0 ± 2.1		2.7 ± 0.3	31 ± 3.2	91 ± 7.4
NaCl	LP	1.6 ± 0.04[a]	28.4 ± 1.4[a]	45.0 ± 2.2	4.1 ± 0.7[a]	4.6 ± 0.1[a]	132 ± 3.2	297 ± 5.2
$N = 12$	ED	2.8 ± 0.26[a]	14.7 ± 1.2[a]	40.9 ± 2.1[a]		3.4 ± 0.9	61 ± 5.3[a]	121 ± 6.0
RB	LP	1.6 ± 0.15[a]	27.8 ± 1.4[a]	44.0 ± 1.8	5.3 ± 1.7[a]	8.6 ± 0.8	133 ± 2.2	294 ± 3.9
$N = 10$	ED	3.3 ± 0.29[a]	11.6 ± 0.6[a]	38.7 ± 2.7[a]		3.5 ± 0.9	60 ± 5.3[a]	133 ± 16.0
$NaHCO_3$	LP	1.9 ± 0.10	24.1 ± 1.6[a]	46.2 ± 2.1	12.1 ± 1.8	30 ± 2.1[a]	93 ± 3.4[a]	283 ± 4.4
$N = 12$	ED	3.1 ± 0.22[a]	10.9 ± 1.0[a]	34.1 ± 2.4[a]		35 ± 2.5[a]	15 ± 1.5[a]	172 ± 15.0[a]
NaAc	LP	1.8 ± 0.06[a]	25.8 ± 1.5[a]	46.1 ± 2.5	11.1 ± 2.3	27 ± 1.4[a]	86 ± 4.1[a]	279 ± 2.2[a]
$N = 12$	ED	3.3 ± 0.20[a]	10.5 ± 0.7[a]	35.0 ± 1.6[a]		32 ± 2.7[a]	9.5 ± 2.4[a]	150 ± 7.6[a]
$NaSO_4$	LP	1.9 ± 0.05[a]	23.9 ± 1.1[a]	44.2 ± 2.1	10.3 ± 1.6	9.1 ± 2.6	94 ± 2.6[a]	271 ± 4.5
$N = 11$	ED	3.8 ± 0.21	9.0 ± 0.7[a]	34.0 ± 1.4[a]		1.6 ± 0.4[a]	12 ± 1.9[a]	174 ± 16[a]

Abbreviations are: LP, late proximal tubule; ED, early distal tubule.
[a] $P < 0.05$ vs. control

Table 5. *R* values for P-D comparisons versus:

	$Flow_{ED}$/ *g kidney wt*	TCO_2ED	$(TF/PCO_2)ED$	Cl_{ED}	$(TF/P_{Cl})ED$	Osm_{ED}	$(TF/P_{Osm})ED$	(Cl_{ED}/Osm_{ED})
Control	0.71[a]	−0.30	0.36	−0.80[a]	−0.69[a]	−0.84[a]	−0.82[a]	−0.003
$N = 8$								
NaCl	0.82[a]	−0.43	−0.46	−0.78[a]	−0.74[a]	−0.52	−0.49	−0.37
$N = 12$								
RB	0.65[a]	0.13	0.0004	−0.82[a]	−0.86[a]	−0.75[a]	−0.77[a]	0.03
$N = 10$								
$NaHCO_3$	0.66[a]	−0.37	−0.24	0.84[a]	0.89[a]	−0.51	0.11	0.53
$N = 12$								
NaAc	0.72[a]	−0.50	−0.51	0.74[a]	0.78[a]	0.40	0.51	0.53
$N = 12$								
Na_2SO_4	0.64[a]	0.03	0.12	0.64[a]	0.65[a]	−0.12	0.54	0.01
$N = 11$								

Abbreviations are: ED, value at early distal tubule; TF/P, tubular fluid/plasma inulin ratio.
[a] significant *P* value ($P < 0.05$)

groups and less than control in the non-chloride expanded groups. Early distal osmolality was significantly greater than control in the non-chloride expanded groups but no different from control in the chloride expanded groups.

Table 5 lists the correlation coefficients (*r*) for the P-D correlated with the different parameters depicted. Each correlation was made with respective parameters within each of the groups studied. All groups showed a significant positive correlation of the P-D with early distal flow–rate. There was no consistant correlation with the late proximal flow–rate (data not shown). P-D could not be significantly correlated with early distal bicarbonate concentration in any group, nor with its tubular fluid to plasma ratio (TF/P). There was a significant correction with tubular fluid osmolality (and its TF/P ratio) in the control and RB groups only. The ratio of early distal tubule chloride concentration to osmolality did not correlate with P-D in any group. Early distal chloride concentration and its respective TF/P ratio (but not absolute chloride delivery nor loop segment transport) correlated significantly with P-D in all groups. The correlation was negative for the control and chloride expanded groups and positive for the non-chloride expanded groups. When all volume expanded animals were combined as a single group, the ED chloride versus P-D correlation was not significant ($r = -0.32$, $P = 0.07$). By contrast, when chloride expanded and non-chloride expanded animals were separated into two groups and the same comparison done within these groups, a significant correlation was obtained for both groups (chloride expanded: $r = -.58$, $P = 0.037$; non-chloride expanded: $r = 0.65$, $P = 0.002$). The direction of the correlation for these two groups was analogous to the directional change of the constituent groups. The regression for the P-D compared to ED flow and ED chloride concentration are shown schematically in Figures 1 and 2, respectively.

Table 6 depicts the values for fluid and sodium retention during infusion of the various experimental solutions expressed as ml/100 g body wt of the animals. As expected, all the expanded animals retained more fluid than the control animals, but that for the Na_2SO_4 was less than the fluid retention for the other expanded groups. Sodium retention was greater than control in all the expanded groups except for Na_2SO_4 in which it was no different from control.

Discussion

The present study demonstrates that equal volume infusion of chloride and non-chloride sodium salts results in greater than

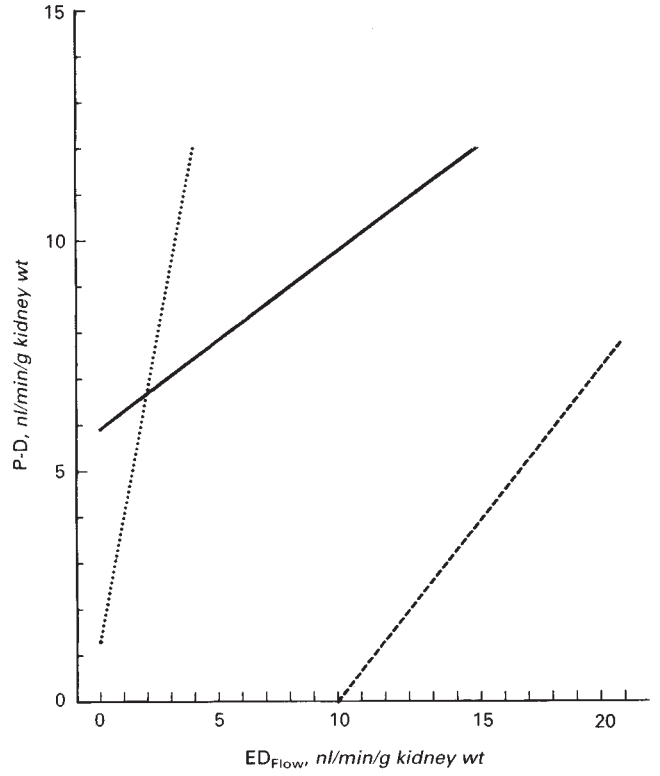

Fig. 1. *P-D versus early distal (ED) flow regression.* Symbols are: control (. . . .) slope = 2.08, *y* intercept = 1.30; Cl expanded (-----) slope = 0.65, $P < 0.05$ vs. control, *y* intercept = −6.7, $P < 0.05$ vs. control; non-Cl expanded (—) slope = 0.40, $P < 0.05$ vs. control, *y* intercept = 5.93, $P < 0.05$ vs. Cl expanded.

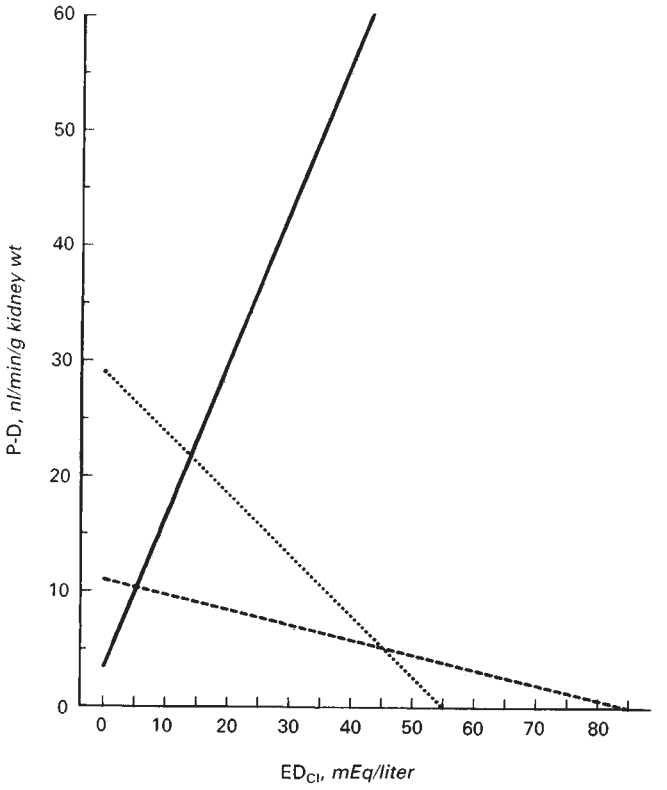

Fig. 2. *P-D versus early distal (ED) chloride concentration.* Symbols are: control (....) slope = 0.54, *y* intercept = 29.5; Cl expanded (----) slope = −0.13, $P < 0.05$ vs. control, *y* intercept = 10.7, $P < 0.05$ vs. controls; non-Cl expanded (———) slope = 1.35, $P < 0.05$ vs. control and Cl expanded, *y* intercept = 4.0, $P < 0.05$ vs. control and Cl expanded.

control whole kidney GFR for the chloride but not for the non-chloride expanded animals. Contributing to this difference was inhibition of TGF as determined by a signficantly smaller than control proximal minus distal nephron SNGFR difference (P-D) for the chloride expanded animals compared to no apparent TGF inhibition in the non-chloride expanded animals (P-D no different from control). Comparing early distal (ED) flow–rate with P-D demonstrated a significantly positive correlation for all groups consistant with stimulation of TGF. Figure 1 demonstrates a significantly smaller slope for the P-D versus ED flow relationship in both the chloride and non-chloride expanded animals compared to control, suggesting decreased sensitivity of the ED flow effect on TGF induced by volume expansion. Comparing early distal chloride concentration with P-D demonstrated a significantly negative correlation for control and chloride expanded animals and a significantly positive correlation for the non-chloride expanded animals. We propose that characteristic intrarenal hormonal environments created by these different models of acute volume expansion contribute to these differences in TGF activation which correlated with ED tubule chloride–concentration.

Variations of ECF or intravascular volume expansion effected by infusion of the various solutions could be responsible for the differences in whole kidney GFR in the various groups. The similar changes in hematocrit and serum protein concentration (final experimental value and decrease from respective initial value) are against any significant differences in degree of volume expansion. Balance studies done during all experiments show higher than control fluid retention (per 100 g body wt) in all the volume expanded animals. The NaCl, RB, $NaHCO_3$, and NaAc animals all had fluid retentions not different from each other, but that for the Na_2SO_4 group was less than the other volume expanded groups, though still greater than control. Sodium retention was higher than control for all expanded groups except Na_2SO_4 in which it was not different from control. Since sodium is primarily an extracellular cation, this data suggests that the ECF was similarly expanded in all volume expanded groups except for the Na_2SO_4 animals. This apparent lack of ECF expansion may have played a role in the relatively low whole kidney GFR seen in the Na_2SO_4 group. This data strongly suggest similar degrees of volume expansion in all expanded groups with the possible exception of the Na_2SO_4 group.

The presence of P-D in these free–flow micropuncture studies depends on the nephrons under the given conditions operating on a responsive portion of their respective feedback response curves. Since the P-D was statistically both greater than zero and correlated with early distal flow–rate for all groups, no group appeared to be operating on an unresponsive portion of their respective curves. Whether the individual volume expanded groups had the same or different flow–response curves cannot be determined from this study.

Table 6. Fluid and Na retention during solution infusions

	Control	NaCl	RB	$NaHCO_3$	NaAc	Na_2SO_4
Fluid *ml/100 g body wt*	5.1 ± 0.65	15.7 ± 0.9[a]	13.5 ± 0.9[a]	13.5 ± 0.9[a]	13.7 ± 1.1[a]	10.4 ± 1.0[a,b]
Na *mEq/100 g body wt*	985 ± 137	2100 ± 158[a]	1888 ± 191[a]	1771 ± 101[a]	1680 ± 126[a]	1170 ± 105[b]

[a] $P < 0.05$ vs. control
[b] $P < 0.05$ vs. NaCl, RB, $NaHCO_3$, and NaAc

The present study suggests ED chloride concentration alone does not determine the direction nor magnitude of the TGF response. Specifically, ED chloride concentration was correlated with opposite influences on TGF in chloride compared to non-chloride volume expanded animals. Although the correlation of ED chloride concentration with TGF for the control and chloride expanded animals was directionally the same, the slope of the P-D versus ED chloride concentration regression (Fig. 2) was significantly less for the chloride expanded compared to the control group. It is proposed that the different environment (hormonal and/or hemodynamic) of the control compared to the volume expanded groups plays a role in how (and how much) the early distal chloride concentration influences the TGF response. Such may also be the case when comparing chloride and non-chloride expanded animals as discussed below. Thus, the present study identifies two parameters (ED flow and chloride concentration) which appear to act as independent signals to initiate TGF, and suggests that a third factor (intrarenal environment) may influence the ultimate response effected by these two signals on TGF.

Since the TGF analysis of the present study was by necessity performed in animals whose kidney was exposed by abdominal surgery, this fact must be considered in evaluating such data and in attempting to make extrapolations to the intact animal. Significant GFR effects were demonstrated by the clearance studies before and after flank incision (FI) detailed in Table 3. Surgery increases sympathetic tone [25], and this increased tone can lead to increased renin and angiotensin production [26]. Thus, these animals undergoing abdominal surgery likely have increased angiotensin II (Ang II) levels compared to the intact animals. In addition, high-frequency renal nerve stimulation has been shown to increase glomerular capillary afferent (R_A) and efferent (R_E) arteriolar resistances and to decrease the glomerular ultrafiltration coefficient (K_f) in rats [27]. These neural and hormonal changes provide a hemodynamic and hormonal environment different from the intact animal and thus a different environment in which TGF is enacted. In addition, it provides a baseline environment different from that of the intact animal on which systemic manipulations like volume expansion are added. The resulting hemodynamic and hormonal effect of such a manipulation may be different when comparing the intact to the surgically exposed kidney.

Extracellular fluid (ECF) volume-expansion exerts significant changes on the intrarenal hormonal environment. Volume expansion increases prostaglandin release into the urine [28] and papillary homogenates from sodium loaded rats synthesize more PGE_2 than do those from control and salt depleted rats [29]. In addition, prostaglandins attentuate the vasoconstrictor actions of Ang II on the renal microvasculature of volume expanded Munich-Wistar rats [30]. These data suggests that the overall hemodynamic effect on the renal vasculature of abdominal surgery and volume expansion may depend on the resultant interaction between these two and possibly other hormonal systems.

Table 7. Loop segment chloride reabsorption (pEq/min/g kidney weight)

Control	NaCl	RB	$NaHCO_3$	NaAc	Na_2SO_4
1301 ± 188	2229 ± 298[a]	1950 ± 222[a]	1491 ± 146	1392 ± 100	1445 ± 144

[a] $P < 0.05$ vs. control

The hormonal response to sodium salt volume expansion depends on the identity of the anion accompanying the sodium. Kotchen et al have shown that sodium administered as the chloride but not the non-chloride salt lowers plasma renin activity [31, 32], and that this ability to lower plasma renin activity is directly correlated with loop segment (the intervening nephron segment between accessible portions of late proximal and early distal tubules) chloride transport [33, 34]. Table 7 depicts the loop segment chloride-transport for the control and volume expanded groups. The chloride expanded animals had loop segment chloride transport-rates greater than control, but that for the non-chloride loaded animals was no different from control. Extrapolating to the data of Kotchen et al, the data of the present study suggests suppression of renin (and likely Ang II) secretion in the chloride but not in the non-chloride expanded groups.

Recent data of Wilcox et al suggest how TGF responses may vary in different hormonal environments. They have shown in a whole kidney model of TGF [35] that chloride induces an increase in renovascular resistance and a decrease in GFR that is associated with a selective increase in excretion of vasoconstricting prostaglandins (thromboxanes) [19]. In other studies, they have shown that this chloride induced fall in GFR requires a fall in Ang II release by the kidney [36]. Combining their data suggests that at least part of the TGF response involves a local increased release of thromboxanes and decreased release of Ang II. Investigation of the possible effector mechanisms for TGF have supported alterations in R_A [37], R_A and R_E [38], and K_f [39] as being important in leading to the changes in SNGFR promoted by macula densa flows and ion content. Since changes in intrarenal prostaglandins and Ang II may be involved in the TGF response elicited by tubular signals, and considering that these hormones influence some or all of these effector parameters, baseline hormonal levels likely play an important role in determining the ultimate result of changes in the levels of these hormones elicited by tubular signals.

In summary, the present study demonstrates greater than control whole kidney GFR for chloride but not for non-chloride

expanded animals. The chloride expanded animals demonstrated TGF inhibition compared to control but the non-chloride expanded animals did not, suggesting one explanation for the difference in whole kidney GFR's. Early distal chloride concentration correlated with TGF inhibition in chloride expanded animals and with augmentation of TGF in non-chloride expanded animals. This apparent difference in ED chloride concentration effect on TGF may relate to different intrarenal hormonal environments in the various models of sodium salt volume–expansion used in this study.

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